

FORM PTO 1390 (REV 11-2000)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER NY-HUBR 1198-US
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371			U.S. APPLICATION NO. (If known, see 37 CFR 1.5) <b>09/980266</b>
INTERNATIONAL APPLICATION NO. <b>PCT/EP00/05272</b>	INTERNATIONAL FILING DATES <b>7 June 2000</b>	PRIORITY DATE CLAIMED <b>9 June 1999</b>	
TITLE OF INVENTION <b>METHOD FOR PRODUCING AN INJECTABLE MEDICAMENT PREPARATION</b>			
APPLICANT(S) FOR DO/EO/US <b>Felix Kratz</b>			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
<ol style="list-style-type: none"> <li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing 35 U.S.C. 371</li> <li>3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371 (f)). The submission must include items (5), (6), (9) and (21) indicated below.</li> <li>4. <input type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).</li> <li>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371 (c)(2)) <ol style="list-style-type: none"> <li>a. <input checked="" type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau).</li> <li>b. <input checked="" type="checkbox"/> has been communicated by the International Bureau.</li> <li>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li>6. <input checked="" type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371 (c)(2)). <ol style="list-style-type: none"> <li>a. <input checked="" type="checkbox"/> is attached hereto.</li> <li>b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4).</li> </ol> </li> <li>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3)) <ol style="list-style-type: none"> <li>a. <input checked="" type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau).</li> <li>b. <input type="checkbox"/> have been communicated by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li>d. <input type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li>8. <input checked="" type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).</li> <li>9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).</li> <li>10. <input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).</li> </ol>			
<b>Items 11 to 20 below concern document(s) or information included:</b>			
<ol style="list-style-type: none"> <li>11. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</li> <li>12. <input checked="" type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li>13. <input checked="" type="checkbox"/> A FIRST preliminary amendment.</li> <li>14. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</li> <li>15. <input type="checkbox"/> A substitute specification.</li> <li>16. <input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li>17. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.</li> <li>18. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4).</li> <li>19. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).</li> <li>20. <input checked="" type="checkbox"/> Other items or information: PCT/ISA/210; PCT/IPEA/409</li> </ol>			

FOUO 11-2000

1990

U.S. APPLICATION NO. (if known, see 37 CFR 1.51) <div style="font-size: 2em; font-weight: bold; text-align: center;">09/980266</div>		INTERNATIONAL APPLICATION NO. PCT/EP00/05272		ATTORNEY'S DOCKET NUMBER HUBR-1198																																									
17. <input checked="" type="checkbox"/> The following fees are submitted: <b>BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):</b> <div style="margin-left: 20px;"><input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO .....\$1040.00 <input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO .....\$890.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO .....\$740.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) .....\$710.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) .....\$100.00</div> <div style="text-align: center; margin-top: 10px;"><b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b></div> <div style="margin-left: 20px;">Surcharge of \$ _____ for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).</div>				<b>CALCULATIONS</b> PTO USE ONLY <table border="1" style="width:100%; border-collapse: collapse;"><tr><td style="width:50%; height: 100px; vertical-align: bottom;">\$ 890.00</td><td style="width:50%;"></td></tr><tr><td>\$</td><td></td></tr><tr><td colspan="2" style="text-align: center;"><b>TOTAL OF ABOVE CALCULATIONS =</b></td></tr><tr><td>\$ 890.00</td><td></td></tr><tr><td colspan="2"><input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above Are reduced by 1/2.</td></tr><tr><td>\$ 445.00</td><td></td></tr><tr><td colspan="2" style="text-align: center;"><b>SUBTOTAL =</b></td></tr><tr><td>\$ 445.00</td><td></td></tr><tr><td colspan="2">Processing fee of \$ _____ for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +</td></tr><tr><td>\$</td><td></td></tr><tr><td colspan="2" style="text-align: center;"><b>TOTAL NATIONAL FEE =</b></td></tr><tr><td>\$ 445.00</td><td></td></tr><tr><td colspan="2">Fee for recording the enclosed assignment (37 CFR 1.21 (h)). Assignment Must be accompanied by appropriate cover sheet (37 CFR 3.28, 3.31) ( _____ per property). +</td></tr><tr><td>\$ 40.00</td><td></td></tr><tr><td colspan="2" style="text-align: center;"><b>TOTAL FEES ENCLOSED =</b></td></tr><tr><td>\$ 485.00</td><td></td></tr><tr><td colspan="2" style="text-align: right;"><b>Amount to be Refunded:</b></td></tr><tr><td>\$</td><td></td></tr><tr><td colspan="2" style="text-align: right;"><b>Charged:</b></td></tr><tr><td>\$</td><td></td></tr></table>		\$ 890.00		\$		<b>TOTAL OF ABOVE CALCULATIONS =</b>		\$ 890.00		<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above Are reduced by 1/2.		\$ 445.00		<b>SUBTOTAL =</b>		\$ 445.00		Processing fee of \$ _____ for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +		\$		<b>TOTAL NATIONAL FEE =</b>		\$ 445.00		Fee for recording the enclosed assignment (37 CFR 1.21 (h)). Assignment Must be accompanied by appropriate cover sheet (37 CFR 3.28, 3.31) ( _____ per property). +		\$ 40.00		<b>TOTAL FEES ENCLOSED =</b>		\$ 485.00		<b>Amount to be Refunded:</b>		\$		<b>Charged:</b>		\$	
\$ 890.00																																													
\$																																													
<b>TOTAL OF ABOVE CALCULATIONS =</b>																																													
\$ 890.00																																													
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above Are reduced by 1/2.																																													
\$ 445.00																																													
<b>SUBTOTAL =</b>																																													
\$ 445.00																																													
Processing fee of \$ _____ for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +																																													
\$																																													
<b>TOTAL NATIONAL FEE =</b>																																													
\$ 445.00																																													
Fee for recording the enclosed assignment (37 CFR 1.21 (h)). Assignment Must be accompanied by appropriate cover sheet (37 CFR 3.28, 3.31) ( _____ per property). +																																													
\$ 40.00																																													
<b>TOTAL FEES ENCLOSED =</b>																																													
\$ 485.00																																													
<b>Amount to be Refunded:</b>																																													
\$																																													
<b>Charged:</b>																																													
\$																																													
a. <input checked="" type="checkbox"/> A check in the amount of \$ <u>485.00</u> to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required or credit any overpayment to my Deposit Account No. <u>50-0624</u> . A duplicate copy of this sheet is enclosed.				<div style="margin-top: 20px;"><b>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.</b></div> <div style="margin-top: 20px;">SEND ALL CORRESPONDENCE TO James R. Crawford FULBRIGHT &amp; JAWORSKI L.L.P. 666 Fifth Avenue New York, New York 10103 (212) 318-3148 Customer No. 24972</div>																																									
<div style="display: flex; justify-content: space-between; align-items: center;"><div>SIGNATURE:  NAME: James R. Crawford</div><div style="text-align: right;">39,155</div></div>																																													

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicants : Kratz  
Serial No. : To Be Assigned  
Filing Date : Herewith  
For : METHOD FOR PRODUCING AN INJECTABLE  
MEDICAMENT PREPARATION

November 30, 2001

Hon. Commissioner of Patents  
and Trademarks  
Washington, D.C. 20231

**PRELIMINARY AMENDMENT**

SIR:

Prior to prosecution, please amend the above-identified patent application as follows:

**IN THE CLAIMS:**

Cancel claims 1-19 and substitute the following claims:

20. Process for producing an injectable medicament preparation, which comprises at least one of a therapeutically effective substance or diagnostically effective substance which is dissolved in an injectable carrier liquid, wherein the diagnostically effective substance is of a compound which comprises an active compound and at least one covalently protein-binding molecular residue which are linked by a spacer, in which the spacer, or the bond between the active compound and the spacer, can be cleaved hydrolytically or enzymically in the body of a subject in a pH-dependent manner.
21. Process according to Claim 20, wherein the spacer, or the bond between the active compound and the spacer, can be cleaved in the body of the subject, with the release of the active compound or of a derivative of the active compound.
22. Process according to Claim 20, wherein the active compound is acytostatic agent, a cytokine, an immunosuppressive agent, a virostatic agent, an antirheumatic agent, an

analgesic, an antiinflammatory agent, an antibiotic, an antimicrobial agent, a signal transduction inhibitor, an angiogenesis inhibitor or a protease inhibitor.

23. Process according to Claim 20, wherein the active compound is selected from the group consisting of anthracyclines, nitrogen mustard derivatives, alkylating agents, purine or pyrimidine antagonists, folic acid antagonists, taxanes, camptothecins, podophyllotoxin derivatives, vinca alkaloids, calicheamicins, maytansinoids or cisconfigured platinum (II) complexes.
24. Process according to claim 20, wherein the diagnostically effective substance possesses one or more radionuclides, one or more ligands comprising radionuclides, one or more position emitters, one or more NMR contrast agents, one or more fluorescent compound(s) or one or more contrast agents in the near IR range.
25. Process according to claim 20, wherein the protein-binding molecule is a maleimide group, a haloacetamide group, a haloacetate group, a pyridylthio group, an N-hydroxysuccinimide ester group, an isothiocyanate group, a disulphide group, a vinylcarbonyl group, an aziridine group or an acetylene group, which can, where appropriate, be substituted.
26. Process according to claim 20, wherein the spacer is an organic molecular residue, which contains at least one aliphatic carbon chain, or an aliphatic carbon ring having 1-12 carbon atoms, some of which can be replaced with oxygen, or at least one aromatic moiety, which can, where appropriate, be substituted.
27. Process according to claim 20, wherein the bond between the active compound and the spacer or the protein-binding molecular residue contains at least one peptide bond.
28. Process according to claim 20, further comprising a carrier molecule.
29. Process according to Claim 28, wherein the carrier molecule and the therapeutically or diagnostically effective substance are brought into contact ex vivo.

30. Therapeutically or diagnostically effective substance comprising at least one active compound, at least one protein-binding molecular residue which is linked to the active compound through a spacer, with the spacer, or the bond between the spacer and the active compound, being cleavable hydrolytically or enzymically in the body in a pH-dependent manner, and with the active compound not being a cytostatic agent.
31. Diagnostically effective substance containing at least one diagnostic agent, wherein at least one protein-binding molecular residue which is linked to the diagnostic agent by means of a spacer, with the spacer, or the bond between the spacer and the diagnostic agent, being hydrolytically or enzymically cleavable in the body in a pH-dependent manner.
32. A method for treating cancer diseases, virus diseases, autoimmune diseases, acute or chronic inflammatory diseases and diseases which are caused by bacteria, fungi or other microorganisms comprising administering the therapeutically or diagnostically defective substance of claim 30 to a patient.
33. Diagnostic kit, which comprises the protein-binding diagnostically effective substance according to claim 20, pharmaceutically acceptable auxiliary substances, carrier substances or a diluent.
34. A method for detecting cancer diseases, auto immune diseases, acute or chronic inflammatory diseases and diseases which are caused by viruses or microorganisms, or for detecting the carrier molecule and its distribution in the body comprising utilizing the kit of claim 33.
35. Process for producing an injectable medicament preparation, which comprises a diagnostically effective substance which is dissolved in an injectable carrier liquid, wherein the diagnostically effective substance is a compound which comprises a diagnostic agent and at least one protein-binding molecular residue.
36. Process according to Claim 35, wherein the diagnostic agent and the protein-binding molecular residue are linked by a spacer.

37. Process according to Claim 35, wherein the bond between the diagnostic agent and the protein-binding molecular residue or the spacer is not cleavable.
38. Process according to Claim 37, wherein the diagnostic agent and the protein-binding molecular residue are linked to each other by an amide bond.

**REMARKS**

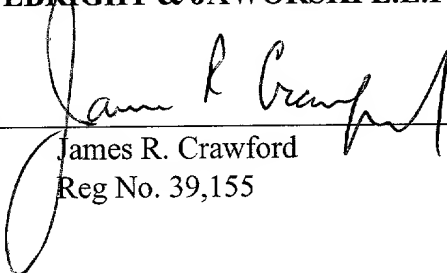
The purpose of this amendment is to conform the claims to standard U.S. practice.

Applicants believe no fee is required for filing these papers. However, the Commissioner is hereby authorized to deduct any fee due, or credit any overpayment to Deposit Account No. 50-0624.

Respectfully submitted,

**FULBRIGHT & JAWORSKI L.L.P.**

By

  
James R. Crawford  
Reg No. 39,155

666 Fifth Avenue  
New York, N.Y. 10103  
(212) 318-3000

Process for producing an injectable medicament  
preparation

Description

5

The invention relates to a process for producing injectable medicament preparations which comprise a therapeutically and/or diagnostically effective substance which consists of an active compound, a  
10 spacer molecule and at least one protein-binding molecule and, after having been brought into contact with the body, binds covalently, by way of the protein-binding molecule, to body fluid constituents or tissue constituents, thereby creating a transport form of the  
15 active compound which can be hydrolytically or enzymically cleaved, in a pH-dependent manner, in the body with the active compound being released.

Most of the drugs which are used at present are low  
20 molecular weight compounds and, after having been administered systemically, exhibit high plasma and total clearances. Furthermore, as a result of diffusion processes, they penetrate into the tissue structures of the body and as a rule have a uniform biodistribution.  
25 These two properties lead to only small quantities of the drug reaching the site of action and, because of its distribution over the healthy tissue of the body, the drug gives rise to side-effects. These disadvantages are particularly pronounced in the case  
30 of those drugs which possess a high cytotoxic potential, such as cytostatic agents, immunosuppressive agents or virostatic agents.

Several strategies are pursued for improving the  
35 selectivity of low molecular weight drugs, for example the chemical derivatization of basic structures, formulation as prodrugs or the coupling of the drugs to carrier molecules. The present invention takes as its starting point those concepts in which drugs have been

09920266-1

chemically bonded to endogenous macromolecules. Conjugates are known in which what are in general cytostatic agents are bound to serum proteins, predominantly to particular carrier molecules such as human serum albumin and human serum transferin, and then administered. These known protein conjugates are prepared either by coupling the cytostatic agent to the serum protein ex vivo in a "one pot method" (DE 41 22 210 A1), and isolating the resulting albumin-cytostatic agent conjugate, or by firstly derivatizing the cytostatic agent with a suitable spacer molecule, isolating the resulting product and, in a second step, coupling the cytostatic agent which has been derivatized in this way to the protein by way of a maleimide group (DE 196 36 889 A1 and PCT/DE 97/02000) and then isolating the resulting albumin-cytostatic agent conjugate. Both methods suffer from the disadvantage that they use plasma proteins, which may contain pathogens. Other disadvantages of the above-described protein-active compound conjugates are their unsatisfactory stability and shelf-life and the technical input required for preparing them.

The invention is based on the object of overcoming these disadvantages. This object is achieved by means of a process for producing an injectable medicament preparation, comprising a therapeutically and/or diagnostically effective substance which is dissolved in an injectable carrier liquid, which process is characterized in that use is made, as the therapeutically and/or diagnostically effective substance, of a compound which consists of an active compound and at least one protein-binding molecular residue which are linked by way of a spacer, in which the spacer, or the bond between the active compound and the spacer, can be cleaved hydrolytically or enzymically in the body in a pH-dependent manner. The active compound, or a derivative of the active compound, is released during the cleavage. An active



compound derivative is understood as meaning substances which include the active compound but which may additionally contain parts of the spacer or of the groups by which the active compound was bonded to the protein-binding molecule. The activity of the active compound should not be impaired as a result of being released as a derivative. The active compound, or its derivative, preferably only displays its activity after having been released.

10

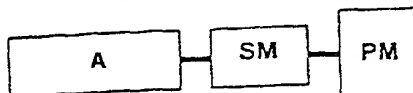
The invention is based on the surprising observation that it is not necessary, as had previously been assumed, to link an active compound to a particular carrier under defined conditions and then to administer the product but that, on the contrary, it is possible to employ therapeutically and/or diagnostically effective substances, which consist of a pharmacological active compound and at least one protein-binding molecular moiety, which are linked to each other by way of a spacer, directly as injectable medicaments since these medicaments, after having been brought into contact with the body, bind covalently, by way of the protein-binding molecule, to body fluid or tissue constituents, predominantly to serum proteins, such that a transport form of the active compound is created in vivo, which transport form reaches the cells or the tissue which is/are the target of the active compound. Since, in the case of the substance which is therapeutically and/or diagnostically effective, the bond in the spacer molecule, or between the active compound and the spacer molecule, can be cleaved, according to the invention, hydrolytically or enzymically in the body in a pH-dependent manner, the active compound is nevertheless released, in a selective manner, at the desired target site.

Because of their protein-binding properties, injectable medicament preparations, which are obtained in accordance with the invention, of therapeutically

09980355 "J" 13001

and/or diagnostically effective substances decisively alter and improve the pharmacokinetic profile of the active compounds. When these therapeutically and/or diagnostically effective substances arrive in body fluids, they bind covalently to body fluid or tissue constituents, preferably to serum proteins, more preferably to serum albumin, in order, in this way, to be present as macromolecular prodrugs which transport the active compound to the target site and/or release it in a metered form.

The therapeutically and/or diagnostically effective substance which is obtained in accordance with the invention consists of an active compound A, a spacer molecule SM and at least one protein-binding molecule PM, having the following general structure:



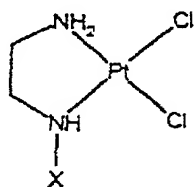
In addition, the substance which is obtained in accordance with the invention can possess labelling groups or labelled elements or molecules, with the substance then being particularly suitable for diagnostic purposes. Preferred labels are one or more radionuclides, one or more ligands comprising radionuclides, one or more positron emitters, one or more NMR contrast agents, one or more fluorescent compound(s) and/or one or more contrast agents in the near IR range.

Within the meaning of this invention, a diagnostic agent is, for example, a labelled active compound as described above or one or more fluorescent compound(s) and/or one or more contrast agents in the near IR range.

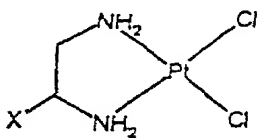
The active compound is a cytostatic agent, a cytokine, an immunosuppressive agent, a virostatic agent, an antirheumatic agent, an analgesic, an antiinflammatory

agent, an antibiotic, an antimycotic agent, a signal transduction inhibitor, an angiogenesis inhibitor or a protease inhibitor.

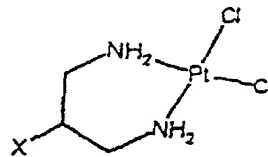
- 5 Cytostatic agents which are preferred for preparing injectable, therapeutically and/or diagnostically effective substances according to the present invention are the anthracyclines doxorubicin, daunorubicin, epirubicin, idarubicin, mitoxantrone and ametantrone, and also related derivatives, the alkylating agents chlorambucil, bendamustin, melphalan and oxazaphosphorins and also related derivatives, the antimetabolites methotrexate, 5-fluorouracil, 5'-deoxy-5-fluorouridine and thioguanine, and also related derivatives, the taxanes paclitaxel and docetaxel, and also related derivatives, the camptothecins topotecan, irinotecan, 9-aminocamptothecin and camptothecin, and also related derivatives, the podophyllotoxin derivatives etoposide, teniposide and mitopodozide, and also related derivatives, the vinca alkaloids vinblastine, vincristine, vindesine and vinorelbine, and also relative derivatives, the calicheamicins, the maytansinoids and a compound of the general formula I to XII:



formula I



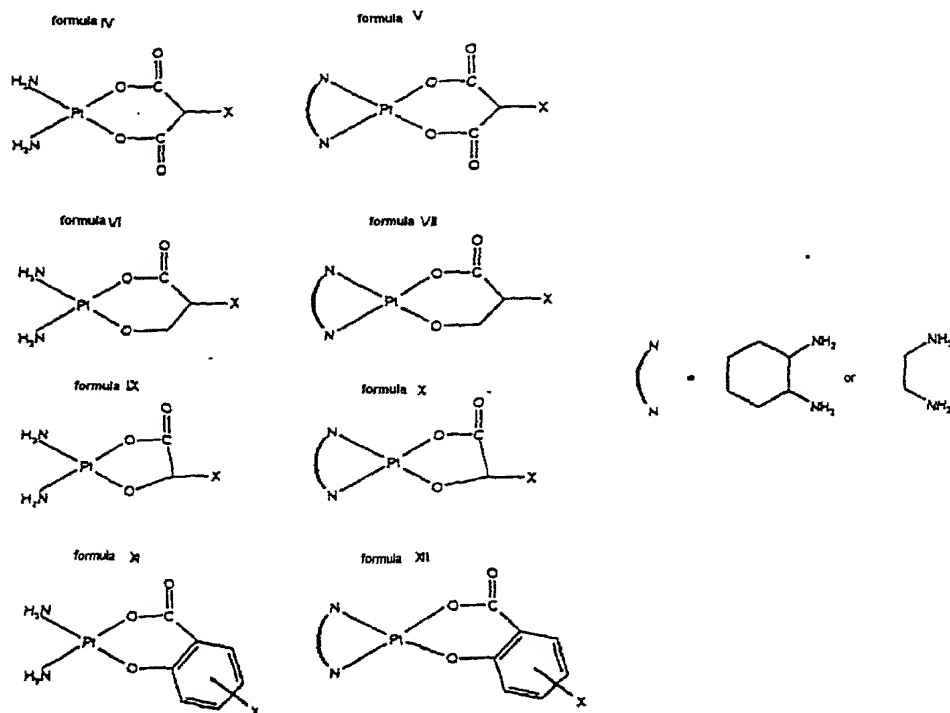
formula II



formula III

where X denotes the spacer molecule SM or the protein-binding molecule PM.

5 Cytokines which are preferred for preparing therapeutically and/or diagnostically effective substances of the present invention are interleukin 2, interferon  $\alpha$ -2a, interferon  $\alpha$ -2b, interferon  $\beta$ -1a,



10 interferon  $\beta$ -1b, interferon  $\gamma$ -1b, and related derivatives. The cytokines employed are as a rule recombinantly produced medicaments.

15 Immunosuppressive agents which are preferred for the process of the present invention are cyclosporin A and related derivatives, and also tacrolimus (FK 506) and related derivatives.

20 Antirheumatic agents which are particularly suitable for the process of the present invention are methotrexate and related derivatives.

Analgesic agents which are preferred for the process of the present invention are salicylic acid derivatives,

such as acetylsalicylic acid and related derivatives,  
drug derivatives which possess an acetic acid or  
propionic acid group, such as diclofenac or  
indomethacin or ibuprofen or naproxen, and aminophenol  
5 derivatives, such as paracetamol.

Antimycotic agents which are preferred for the process  
of the present invention are amphotericin B and related  
derivatives.

10

Virostatic agents which are preferred for the process  
of the present invention are nucleoside analogues, such  
as aciclovir, ganciclovir, idoxuridine, ribavirin,  
vidaribine, zidovudine, didanosine and 2',3'-  
15 dideoxycytidine (ddC), and related derivatives, and  
amantadine.

Antibiotics which are preferred for the process of the  
present invention are sulphonamides, such as  
20 sulanilamide, sulphacarbamide and sulphamethoxydiazine  
and related derivatives, penicillins, such as  
6-aminopenicillanic acid, penicillin G and  
penicillin V, and related derivatives,  
isoxazoypenicillins (e.g. oxacillin, cloxacillin,  
25 flucloxacillin), and related derivatives,  $\alpha$ -substituted  
benzylpenicillins (e.g. ampicillin, carbenicillin,  
pivampicillin and amoxicillin), and related  
derivatives, acylaminopenicillins (e.g. mezlocillin,  
azlocillin, piperacillin and apalicillin), and related  
30 derivatives, amidinopenicillins, such as mecillinam,  
atypical  $\beta$ -lactams, such as imipenam and aztreonam,  
cephalosporins, such as cefalexin, cefradine, cefaclor,  
cefadroxil, cefixime, cefpodoxime, cefazolin,  
cefazedone, cefuroxime, cefamandole, cefotiam,  
35 cefoxitin, cefotetan, cefmetazole, latamoxef,  
cefotaxime, ceftriaxone, ceftizoxime, cefmonoxime,  
ceftazidime, cefsulodin and cefoperazone, and related  
derivatives, tetracyclines, such as tetracycline,  
chlortetracycline, oxytetracycline, demeclocycline,

rolitetetracycline, doxycycline and minocycline, and related derivatives, chloramphenicols, such as chloramphenicol and thiamphenicol, and related derivatives, gyrase inhibitors, such as nalixidic acid, 5 pipemidic acid, norfloxacin, ofloxacin, ciprofloxacin and enoxacin, and related derivatives, and tuberculosis agents, such as isoniazid and related derivatives.

09980255 113001  
The spacer molecule SM is an organic molecule which consists of an aliphatic carbon chain and/or an aliphatic carbon ring and/or at least one aromatic compound. The carbon chain/ring preferably consists of 1-12 carbon atoms, some of which may be replaced by oxygen atoms, and can, where appropriate, be 15 substituted, particularly by one or more water-soluble groups, such as sulphonic acid, aminoalkyl or hydroxyl groups. The aromatic compound is preferably a benzene ring which can, where appropriate, be substituted, for example by the abovementioned water-soluble group. To 20 improve its water solubility, the aliphatic carbon chain can contain oxygen atoms and be expediently derived, for this purpose, from an oligoethylene oxide or oligopropylene oxide chain, for example a diethylene glycol, triethylene glycol or dipropylene glycol chain.

25 The protein-binding molecule PM is preferably a maleimide group, a haloacetamide group, a haloacetate group, a pyridyldithio group, an N-hydroxysuccinimide ester group or an isothiocyanate group. It can also be 30 a disulphide group, a vinylcarbonyl group, an aziridine group or an acetylene group. The disulphide group is preferably activated, as a rule by a thionitrobenzoic acid (e.g. 5'-thio-2-nitrobenzoic acid) being the exchangeable group. The groups may be substituted, 35 where appropriate. The maleimide, pyridyldithio or N-hydroxysuccinimide ester group can, where appropriate, be substituted by alkyl or by the abovementioned water-soluble groups. The PM possesses protein-binding properties, i.e., it binds covalently,

in a physiological environment, to particular amino acids on the surface of the protein. In this connection, the maleimide group, the haloacetamide group, the haloacetate group, the pyridyldithio group, the disulphide group, the vinylcarbonyl group, the aziridine group and/or the acetylene group preferably reacts with HS groups of cysteines, while the N-hydroxysuccinimide ester group and the isothiocyanate group preferably react with the amino group of lysines, on the surface of the protein.

Following parenteral administration, the therapeutically and/or diagnostically effective substance, which is prepared, as an injectable medicament preparation, by the process of the present invention, arrives in the blood stream and can bind to proteins by way of the PM. The binding preferably takes place to serum proteins, in particular serum albumin. It has been found that, in a physiological environment, the therapeutically and/or diagnostically effective substance reacts, by way of the maleimide group, the haloacetamide group, the haloacetate group, the pyridyldithio group, the disulphide group, the vinylcarbonyl group, the aziridine group or the acetylene group, with the free cysteine-34 of the albumin, in particular, and is in this way bound covalently. Pharmacologically active substances having an N-hydroxysuccinimide ester group or isothiocyanate group preferentially bind to the  $\epsilon$ -amino group of lysines on the protein surface of albumin or other serum proteins. Serum proteins, such as albumin or transferrin, have a markedly long half-life in the systemic circulation (up to 19 days - Peters, T. Jr. (1985): Serum albumin. Adv. Protein. Chem. 37, 161-245). Because the permeability to macromolecules of the walls of the vessels in the malignant, infected or inflamed tissue is increased, the serum albumin preferentially makes its way into this target tissue (Maeda, H.; Matsumura, Y. Crit. Rev. Ther. Drug Carrier

06980265 "413004

Sys. (1989), 6, 193-210). As a result, an active compound which is coupled to albumin is able to reach the site of action in a more targeted manner. Furthermore, the covalent coupling of the active compound to serum proteins in the blood stream prevents the active compound from diffusing into healthy tissue structures in the body or being eliminated by way of the kidneys, or damaging these tissues to the same extent as would the unbound active compound. As a result, the pharmacokinetic profile of the active compound is altered and improved since its effect is increased as a result of it being enriched at the site of action and, at the same time, the toxic effects on healthy systems in the body are diminished.

The therapeutically and/or diagnostically effective substances which are used in accordance with the present invention contain a defined chemical bond in the spacer molecule or between the SM and the A. This bond can be cleaved or hydrolytically cleaved in a pH-dependent manner, preferably in an acid-labile manner, or else it contains a bond which can be cleaved enzymically in the body, preferably a peptide bond.

Examples of bonds which are cleaved by hydrolysis, with the active compound being released, are ester bonds or metal complex bonds, as are present in platinum-dicarboxylate complexes, with a diamminediaquo-platinum(II) complex being released. The acid-labile cleavable bonds are acetal, ketal, imine, hydrazone, carboxylhydrazone or sulphonylhydrazone bonds, or cis-aconityl bonds or bonds containing a trityl group, with it being possible for the trityl group to be substituted or unsubstituted. Preferred therapeutically/diagnostically relevant acid-labile bonds are described, for example, in Kratz et al. (1990) Crit. Rev. Ther. Drug. Car. Sys. 16 (3), 245-288. The peptide sequence in the peptide bonds which are formed consists as a rule of about 2-30 amino



acids. In this connection, the peptide sequence is preferably tailor-made for the substrate specificity of particular enzymes, which are designated target enzymes in that which follows, such that the peptide sequence, or a part of this sequence, is recognized by an enzyme in the body and the peptide is cleaved. According to another embodiment of the present invention, the enzymically cleavable bond consists of a bond which is not a peptide bond. Examples are carbamate bonds, which, by the mediation of disease-specific enzymes, e.g. glutathione-S-transferases, glucuronidases and galactosidases, release the active compound or an active compound derivative. It is also readily possible for an enzymically cleavable bond to be composed of a peptide sequence and one of the abovementioned bonds which is not a peptide bond. The target enzymes can be either endogenous enzymes or enzymes which occur in microorganisms or are formed by microorganisms.

The target enzymes are as a rule proteases, serine proteases, plasminogen activators and peptidases, for example matrix metalloproteases (MMP) or cysteine proteases, which are formed to an increased extent, or are activated, in association with diseases such as rheumatoid arthritis or cancer, thereby leading to excessive tissue breakdown, to inflammations and to metastases. Target enzymes are, in particular, MMP 2, MMP3 and MMP 9, which are involved, as proteases, in the abovementioned pathological processes (Vassalli, J., Pepper, M.S. (1994), *Nature* 370, 14-15, Brown, P.D. (1995), *Advan Enzyme Regul.* 35, 291-301).

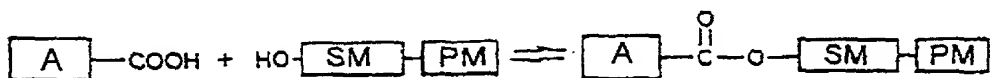
Further proteases which constitute target enzymes for therapeutically and/or diagnostically effective substances of the present invention are cathepsins, in particular cathepsin B, H and L, which have been identified as key enzymes in inflammatory and malignant diseases.

The abovementioned bond types ensure that the active compound, or a correspondingly active derivative, is released, extracellularly and/or intracellularly, following uptake of the conjugate by the cell, and can express its therapeutic and/or diagnostic effect, at the active site.

The cleavage can also take place in such a way that it is not the active compound as such which is cleaved off, but, instead, a derivative of the active compound. Such a derivative constantly contains the active compound and groups which are bonded to it and which are derived from the spacer molecule, depending on the site at which the desired cleavage has taken place.

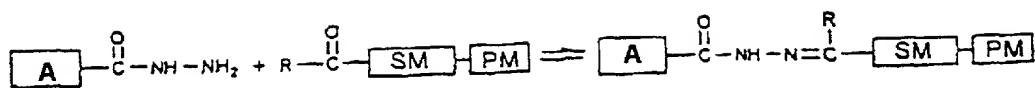
The therapeutically and/or diagnostically effective substance which is used in the process of the present invention can be prepared in accordance with one of the general descriptions given below:

Active compounds which possess a COOH group are derivatized in the following manner:



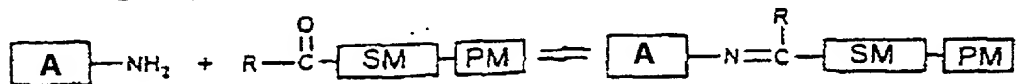
In this connection, the esterification is effected using customary methods with which the skilled person is familiar.

It is furthermore possible to convert the COOH group into a hydrazide group, for example by reacting with tert-alkyl carbazates and subsequently cleaving with acids (described in DE 196 36 889), and to react the drug possessing a hydrazide group with a spacer which consists of PM and SM and which contains a carbonyl component, as described, inter alia, in DE 196 36 889 A1 and also PCT/DE 97/02000:



R = H, alkyl, phenyl or substituted phenyl

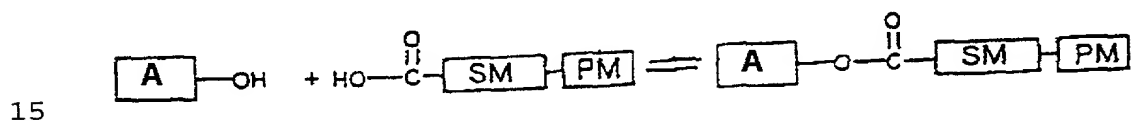
Active compounds of the present invention which possess an NH<sub>2</sub> group are derivatized in the following manner:



R = H, alkyl, phenyl or substituted phenyl

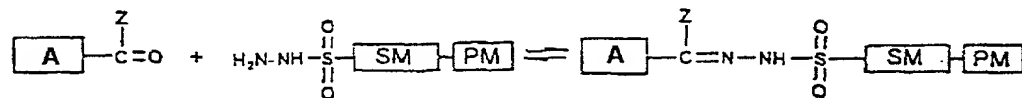
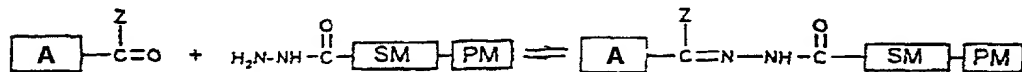
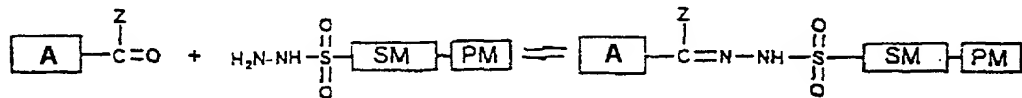
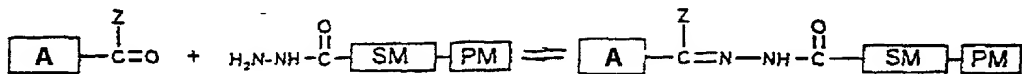
In this connection, the reaction to give the imine derivatives is effected using customary methods with which the skilled person is familiar.

Active compounds of the present invention which possess an OH group are derivatized in the following manner:



In this connection, the esterification is effected using customary methods with which the skilled person is familiar.

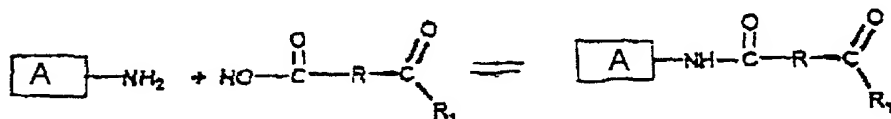
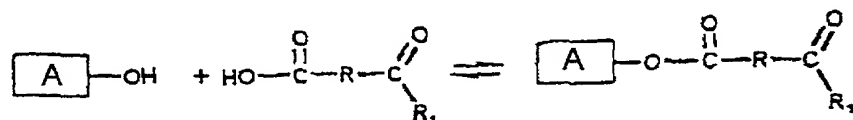
Active compounds of the present invention which possess a carbonyl component are derivatized in the following manner:



Z = chemical group belonging to the active compound

In this connection, the reaction to give the carboxyhydrazone, sulphonylhydrazone, hydrazone and/or imine derivatives takes place in accordance with methods which are described, inter alia, in DE 196 36 889 A1 and/or PCT/DE 97/02000, or using customary methods with which the skilled person is familiar.

It is furthermore possible to convert an OH group or an NH<sub>2</sub> group on an active compound into a carbonyl component, for example by means of esterification or amide formation using a carboxylic acid-carrying carbonyl component in accordance with the following general formula:



where R is an aliphatic carbon chain and/or an aliphatic carbon ring and/or an aromatic moiety and R<sub>1</sub> = H, or an alkyl, phenyl or substituted phenyl group. As a rule, R consists of from 1 to 12 carbon atoms which may, where appropriate, be substituted, e.g. by water-soluble groups such as sulphonic acid, aminoalkyl or hydroxyl groups. The aromatic moiety is as a rule a benzene ring which can, where appropriate, be substituted, for example by the abovementioned water-soluble groups.

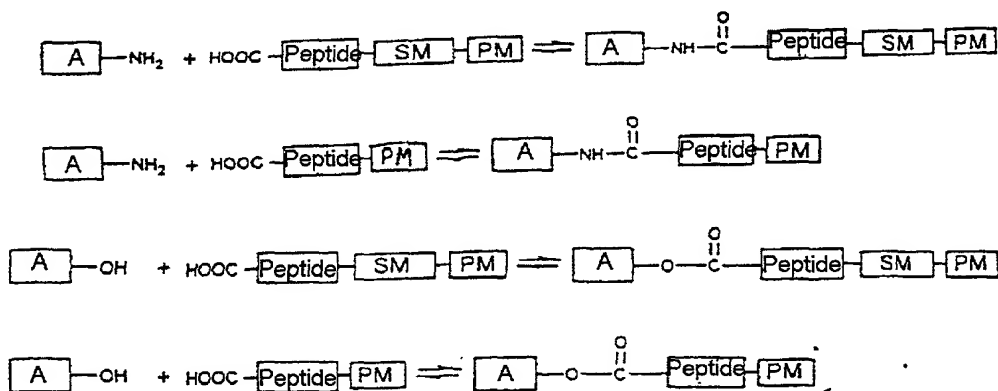
The carbonyl component can furthermore be introduced using other chemical reactions, for example by means of an electrophilic substitution at the HO or NH<sub>2</sub> group in the active compound using a suitable carbonyl component.

The active compounds which have been derivatized in this way, and which now possess a carbonyl component, are reacted, in analogy with the above-described methods, with the protein-binding spacer molecules, which possess an amino, hydrazide or hydrazine group, to give the corresponding carboxylhydrazone, sulphonylhydrazone, hydrazone or imine derivatives. The acid-labile cleavage of these bonds consequently leads to a release of the derivatized active compound which possesses a carbonyl component.

The spacers, which consist of the protein-binding molecule PM and the spacer molecule SM, can be prepared, for example, in accordance with methods which are described, inter alia, in DE 196 36 889 A1, U. Beyer et al. *Chemical Monthly*, 128, 91, 1997, R.S. Greenfield et al., *Cancer Res.*, 50, 6600, 1990, T. Kaneko et al., *Bioconjugate Chem.*, 2, 133, 1991, *Bioconjugate Techniques*, G.T. Hermanson, Academic Press, 1996 or in US Patent 4,251,445.

Therapeutically and/or diagnostically effective substances for the present invention which contain a peptide bond can be prepared by reacting a peptide, which consists of from 2 to about 30 amino acids, with a protein-binding compound such that a protein-binding molecule is introduced directly, or by way of a spacer molecule SM, at the N-terminal end of the peptide. Such protein-binding peptide derivatives are preferably synthesized using a solid phase synthesis with which the skilled person is familiar, with a carboxylic acid-carrying, protein-binding spacer molecule, for example a maleimidocarboxylic acid, being bonded, by means of peptide coupling, to the N-terminal end of the peptide in the last step of the peptide synthesis and the protein-binding peptide then being eliminated from the solid phase. The peptide derivatives which are obtained in this way can be reacted with an active compound, which possesses an  $\text{NH}_2$  or OH group, in the presence of

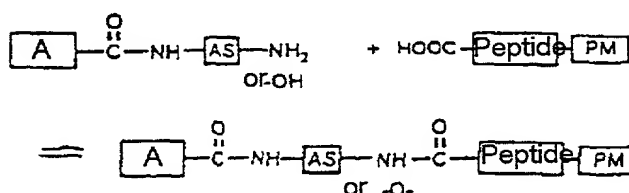
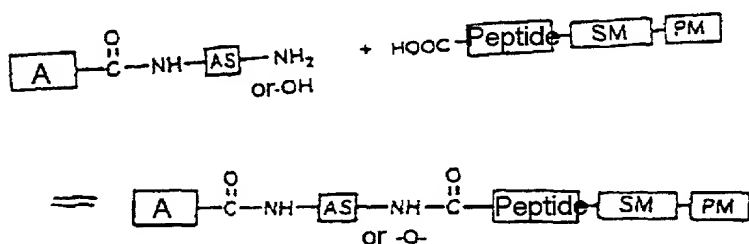
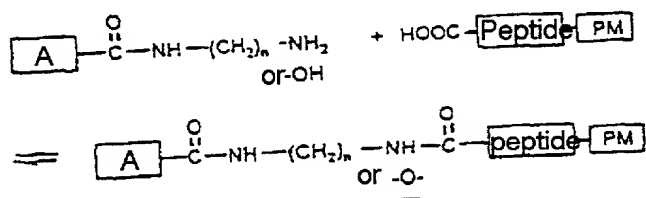
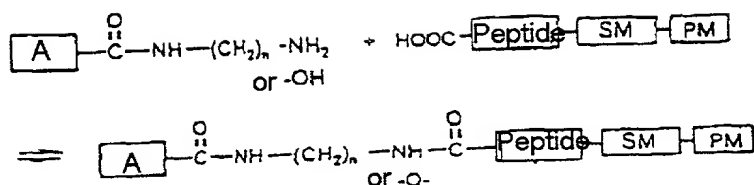
a condensing agent, such as N,N'-dicyclohexylcarbodiimide (DCC) or N-cyclohexyl-N'-(2-morpholinoethyl)carbodiimide metho-p-toluenesulphonate (CMC), (benzotriazol-1-yloxy)trispyrrolidinophosphonium hexafluorophosphate (pyBOP) or O-benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate, and, where appropriate, with the addition of N-hydroxysuccinimide or of a water-soluble N-hydroxysuccinimide, such as N-hydroxysuccinimide-3-sulphonic acid sodium salt or 1-hydroxybenzotriazole and/or in the presence of a base, such as N-methylmorpholine or triethylamine, to give the corresponding protein-binding active compound-peptide derivatives:



15

It is furthermore possible to introduce an NH<sub>2</sub> or OH group by way of the COOH group which is present on the active compounds, for example by means of derivatizing with the amino acids (AA) lysine, serine or threonine by way of their α-amino group or by way of the α-amino group using a diamino compound of the general formula H<sub>2</sub>N-(CH<sub>2</sub>)<sub>n</sub>-NH<sub>2</sub> or using an alcoholamine of the general formula H<sub>2</sub>N-(CH<sub>2</sub>)<sub>n</sub>-OH, where n=1-12, and subsequently reacting these derivatives with the abovementioned peptide derivatives to give the corresponding protein-binding active compound-peptide derivatives:

TOP SECRET 992866



5

AA = lysine, serine or threonine

10 The substrate specificity of target enzymes, for example of MMP 2, MMP3 and MMP 9 and cathepsin B, H and L, is known (Netzel-Arnett et al. (1993), *Biochemistry* 32, 6427-6432, Shuja S., Sheahan, K., Murnane, M.J. (1991), *Int. J. Cancer* 49, 341-346, Lah, T.T., Kos, J. (1998), *Biol. Chem.* 379, 125-130).

15 For example, in the case of MMP 2 and MMP 9, octapeptides (P<sub>4</sub>-P'<sub>4</sub>) have been identified, which octapeptides simulate the cleavage sequence of the

collagen chain and are cleaved particularly efficiently by MMP 2 and 9:

5

Peptide

P<sub>4</sub> P<sub>3</sub> P<sub>2</sub> P<sub>1</sub> P'<sub>1</sub> P'<sub>2</sub> P'<sub>3</sub> P'<sub>4</sub>

Gly-Pro-**Leu**-Gly-Ile-Ala-Gly-Gln

Gly-Pro-Gln-Gly-Ile-**Trp**-Gly-Gln

10 (Netzel-Arnett et al., *Biochemistry* 32, 1993, 6427-6432).

The peptides are enzymically cleaved exclusively at the P<sub>1</sub> - P'<sub>1</sub> bond.

15

Furthermore, substrate-specific dipeptides having the sequence -Arg-Arg-, -Phe-Lys-, Gly-Phe-Leu-Gly, Gly-Phe-Ala-Leu and Ala-Leu-Ala-Leu are known in the case of cathepsin B (Werle, B., Ebert, E., Klein, W., Spiess, E. (1995), *Biol. Chem. Hoppe-Seyler* 376, 157-164; Ulricht, B., Spiess, E., Schwartz-Albiez, R., Ebert, W. (1995), *Biol. Chem. Hoppe-Seyler* 376, 404-414).

20

25 The peptide sequence which contains the expected peptide cleavage site which is relevant for the target enzyme can also be constructed such that the expected peptide cleavage site is repeated several times, for example by means of:

30

-Gly-Pro-**Leu**-Gly-Ile-Ala-Gly-Gln-Gly-Pro-**Leu**-Gly-Ile-Ala-Gly-Gln

or

35

-Phe-Lys-Phe-Lys-Phe-Lys-Phe-Lys-Phe-Lys-Phe-Lys-

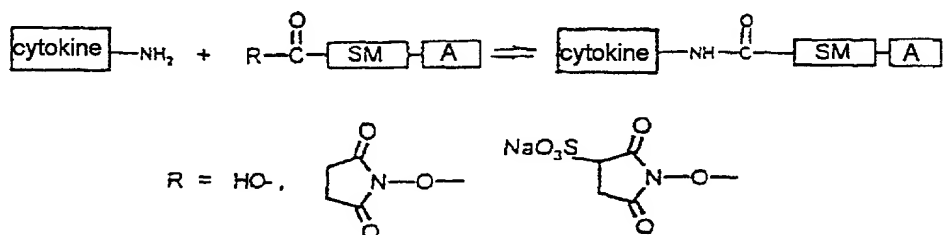
or it is possible to integrate a repetitive peptide sequence which increases the distance between the

10980556 11001



-Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly-Phe-  
5 Lys-Phe-Lys-

Therapeutically and/or diagnostically effective substances for the present invention which contain a cytokine can be prepared by reacting the cytokine with  
20 a protein-binding group-containing spacer molecule which possesses a carboxylic acid or an activated carboxylic acid:



If the spacer molecule possesses an N-hydroxysuccinimide ester group (N-hydroxysuccinimide or N-hydroxysuccinimide-3-sulphonic acid sodium salt), it is reacted directly with the cytokine. The reaction of the cytokine with a protein-binding group-containing spacer molecule which possesses a carboxylic acid takes place in the presence of a condensing agent, such as N,N'-dicyclohexylcarbodiimide (DCC) or N-cyclohexyl-N'-(2-morpholinoethyl)carbodiimide metho-p-toluene-sulphonate (CMC), and, where appropriate, with addition of N-hydroxysuccinimide or N-hydroxysuccinimide-3-

0958066 44001  
sulphonic acid sodium salt, to give the corresponding  
protein-binding cytokine derivatives. The cytokines  
which have been derivatized in this way are expediently  
purified by means of gel filtration chromatography. The  
5 skilled person is familiar with the above-described  
reactions (Bioconjugate Techniques, G.T. Hermanson,  
Academic Press, 1996).

Following the synthesis of the therapeutically and/or  
10 diagnostically effective substance, an injectable  
medicament preparation, which comprises the  
therapeutically and/or diagnostically effective  
substance, is produced in a suitable carrier liquid.  
The therapeutically and/or diagnostically effective  
15 substance is preferably present as a lyophilisate, with  
it being possible to add customary carriers and/or  
pharmaceutical auxiliary substances, such as  
polysorbates, glucose, lactose, mannose, citric acid,  
tromethamol, triethanolamine or aminoacetic acid,  
20 before or after the lyophilisation. The injectable  
medicament preparation must be produced in such a way  
that the protein-binding molecule is not deactivated,  
eliminated or hydrolysed by the process of dissolving  
in the injectable carrier liquid. Furthermore, care  
25 must be taken to ensure that the acid-labile bond in  
the therapeutically and/or diagnostically effective  
substance, which bond is an ester, acetal, ketal,  
imine, hydrazone, carboxylhydrazone or sulphonyl-  
hydrazone bond, is not hydrolysed. The protein-binding  
30 molecules which are used within the context of the  
present invention are base-sensitive, which means that  
the pH of the carrier liquid should not exceed a pH of  
8.0. The pH is preferably in the range of pH 4.0-7.0,  
more preferably between pH 6.0 and pH 7.0. In addition,  
35 the carrier liquid must naturally be physiologically  
tolerated.

Carrier liquids which are preferred are virtually  
isotonic salt buffers, for example phosphate, acetate

or citrate buffers, such as 0.004 M sodium phosphate, 0.15 M NaCl, pH 6.0-7.0, or 0.01 M sodium acetate, 0.14 M NaCl, pH 5.0-6.5. The carrier liquid used can also be an isotonic sodium chloride solution. The salt  
5 buffers may contain customary excipients and/or auxiliary substances, such as polysorbates, glucose, lactose, mannose, citric acid, tromethamol, triethanolamine or aminoacetic acid.

- 10 The solubility of the therapeutically and/or diagnostically effective substance in the injectable carrier liquid can be improved by means of pharmaceutical solvents, such as ethanol, isopropanol, 1,2-propylene glycol, glycerol, macrogols, polyethylene  
15 glycols and/or polyethylene oxides, or by means of solubility promoters, e.g. Tween, cremophore or polyvinylpyrrolidone. For this purpose, the therapeutically and/or diagnostically effective substance is either dissolved in the pharmaceutical  
20 solvent or solubility promoter and then diluted with a salt buffer, or else a carrier liquid, which contains the salt buffer and at least one pharmaceutical solvent or solubility promoter, is used directly for dissolving the therapeutically and/or diagnostically effective  
25 substance. In this connection, the concentration of the pharmaceutical solvents and/or solubility promoters do [sic] not exceed the quantities stipulated by the Arzneimittelgesetz (AMG) (Medicines Act).
- 30 The carrier liquid should preferably be selected such that the process of dissolving the therapeutically and/or diagnostically effective substance in the carrier liquid is concluded after a few minutes, such that an injectable medicament preparation is made  
35 available at the patient's bedside.

It is also possible, in addition, for a carrier molecule, such as one of those mentioned at the outset, to be brought into contact with the effective

09980266 113001

substance, with the effective substance being able to bind to this carrier molecule. According to another embodiment, the present invention consequently encompasses the step of bringing the carrier molecule and the protein-binding therapeutically and/or diagnostically effective substance into contact ex vivo and subsequently administering them parenterally. In this way, it is possible, if desired or if necessary, to improve the selectivity of the therapeutically and/or diagnostically effective substance for a carrier molecule, for example for albumin. The carrier molecules are preferably selected from the carrier molecules which have been mentioned, in particular serum proteins.

The therapeutically and/or diagnostically effective substance which has been prepared in accordance with the process according to the invention is consequently suitable for treating cancer diseases, virus diseases, autoimmune diseases, acute or chronic inflammatory diseases and/or diseases which are caused by bacteria, fungi or other microorganisms.

Another part of the subject-matter of the present invention is a therapeutically and/or diagnostically effective substance containing at least one active compound, which substance is characterized in that it possesses at least one protein-binding molecular residue which is linked to the active compound by means of a spacer, with the spacer, or the bond between the spacer and the active compound, being hydrolytically or enzymically cleavable in the body in a pH-dependent manner, resulting in the release of the active compound, with the active compound not being a cytostatic agent.

Yet another part of the subject-matter of the present invention is a diagnostically effective substance containing at least one diagnostic agent, which

substance is characterized in that it [lacuna] at least one protein-binding molecular residue which is linked to the diagnostic agent by means of a spacer, with the spacer, or the bond between the spacer and the  
5 diagnostic agent, being hydrolytically or enzymically cleavable in the body in a pH-dependent manner, resulting in the release of the diagnostic agent. As mentioned above, the diagnostic agent preferably comprises one or more radionuclides, one or more  
10 ligands comprising radionuclides, one or more positron emitters, one or more NMR contrast agents, one or more fluorescent compound(s) and/or one or more contrast agents in the near IR range.

15 Another embodiment of the present invention relates to a diagnostic kit which comprises a protein-binding, diagnostically effective substance according to the invention, where appropriate together with the carrier molecule and pharmaceutically acceptable auxiliary  
20 substances, carrier substances and/or diluents, which are selected, in particular, from those mentioned above. The diagnostic kit according to the invention can preferably be used for detecting the diseases as defined above or for detecting carrier molecules and/or  
25 their distribution in the body.

Yet another part of the subject-matter of the present invention is a process for producing an injectable medicament preparation, comprising a diagnostically  
30 effective substance which is dissolved in an injectable carrier liquid, which process is characterized in that a compound comprising a diagnostic agent and at least one protein-binding molecular residue is used as the diagnostically effective substance. A diagnostic agent  
35 can be one of the abovementioned compounds, for example one or more radionuclides, one or more ligands comprising radionuclides, one or more positron emitters, one or more NMR contrast agents, one or more fluorescent compound(s) and/or one or more contrast

09980266 "113001

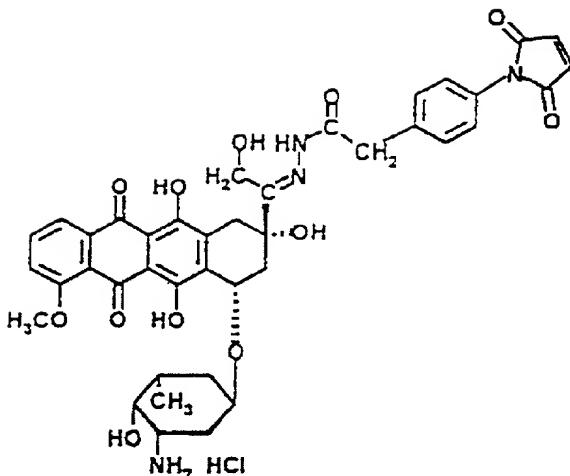
agents in the near IR range. The diagnostic agent and the at least one protein-binding molecular residue can also be linked by means of a spacer. In this case, preference is given to the spacer, or the bond which links the two components, not being cleavable. Examples of bonds which are not cleavable in the body, and which can be present in the case of the bond to the diagnostically effective substance, are amide bonds, saturated or unsaturated carbon-carbon bonds or bonds between carbon and a heteroatom, i.e. -C-X-, where X is preferably O, N, S or P. An amide bond is a preferred bond. Preference is given to the therapeutically effective substance being released since, as a rule, the low molecular weight active compound must interact with its molecular target in order to express its pharmacological activity. In the case of diagnostically effective substances, on the other hand, it is not absolutely necessary for the protein-bound diagnostic agent to be released; however, this may occur. According to the invention, therefore, the diagnostically effective substance can be additionally bound to the spacer molecule by way of a bond which is not cleavable in the body or be bound directly to the protein-binding group without any spacer molecule SM being present.

The following examples explain the invention in more detail.

#### Example 1

##### Preparation of DOXO-HYD

The pharmacologically active substance depicted below (abbreviated to DOXO-HYD) consists of the cytostatic agent doxorubicin, a maleimide group as the protein-binding molecule PM, and a phenylacetylhydrazone spacer as the spacer molecule SM. The bond between doxorubicin and the spacer molecule SM is an acid-labile carboxyl-hydrazone bond:



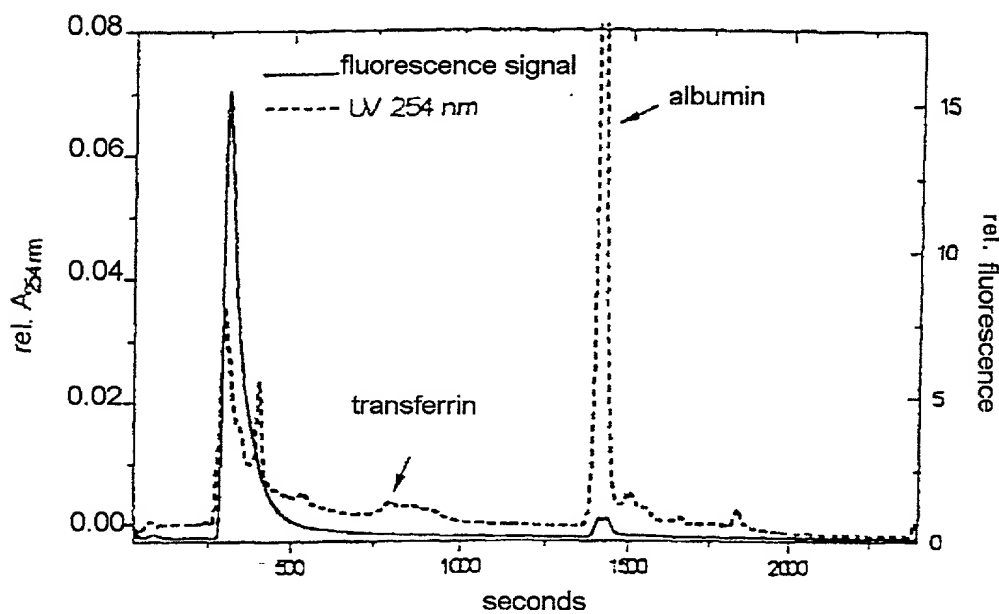
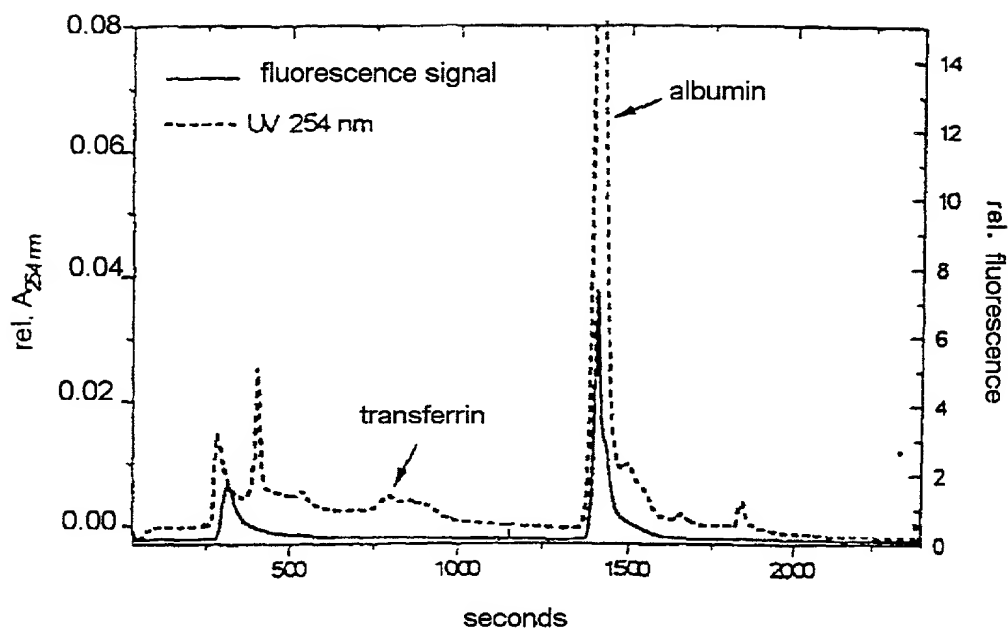
10.51 mg of DOXO-HYD are dissolved, by shaking, in 2.0 ml of 1,2-propylene glycol and this solution is subsequently diluted with 8.0 ml of phosphate buffer (0.004 M sodium phosphate, 0.15 M NaCl - pH 6.5) and homogenized (concentration of DOXO-HYD in the carrier liquid  $\approx 1300 \mu\text{M}$ ). The injectable medicament preparation of DOXO-HYD which had been produced in this way was immediately administered intravenously to experimental animals (see below).

#### Example 2

##### Binding of DOXO-HYD to human plasma

After DOXO-HYD arrives in the blood stream, it binds to serum proteins, preferentially to serumalbumin, such that DOXO-HYD is present, inter alia, as an acid-labile albumin-doxorubicin conjugate.

Studies of the incubation of human blood plasma with DOXO-HYD at  $37^\circ\text{C}$  show that most of the DOXO-HYD is covalently bonded to the albumin after 5 minutes of incubation. (A) - in contrast to free doxorubicin (B). This situation is depicted in the following chromatograms. (A and B):



5

In this experiment, the plasma sample was separated, after the incubation had taken place, on a POROS®-20 anion exchange column (the proteins were detected at 254 nm while the anthracycline was detected by fluorescence).

10

### Example 3

The activity of DOXO-HYD in vivo



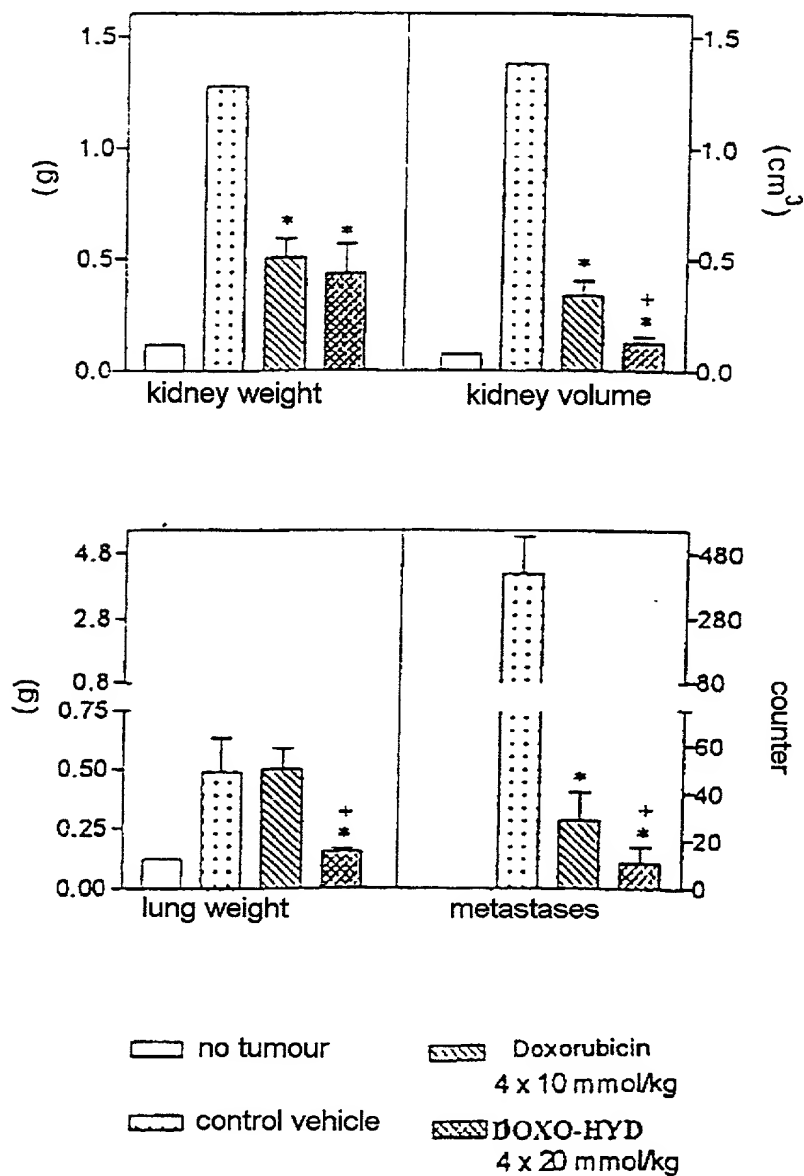
The biological data listed below clarify the in vivo activity of DOXO-HYD as compared with that of free doxorubicin: in what is termed the RENCA (renal cell carcinoma)-model, doxorubicin and DOXO-HYD were compared with each other, at approximately equitoxic doses, as regards their antitumour activity (intravenous therapy 10 days after injecting about 1 million kidney carcinoma cells into the left kidney).

- 10 **Animals:** Balb/c mice, female; **Tumour:** RENCA, renal cell carcinoma  
**Therapy:** Intravenous (i.v.) on day (d) 10, 13, 17 and 20, end of experiment on d24

Number of Mice	Substance	Dose	Average decrease in body weight (%) d 1-24
10	Control		
10	Doxorubicin	4 × 10 mmol/kg	-15
10	DOXO-HYD	4 × 20 mmol/kg	-15

- 15 The results of this experiment are depicted below. DOXO-HYD exhibits very good antitumour activity and achieves a marked reduction in kidney tumour volume and in the number of lung metastases as compared with the  
20 control group and the doxorubicin-treated group.

Weight and volume of the kidneys and kidney tumours and the number of lung metastases



5

\* significant in relation to the control group (control)

+ significant in relation to the group which received doxorubicin.

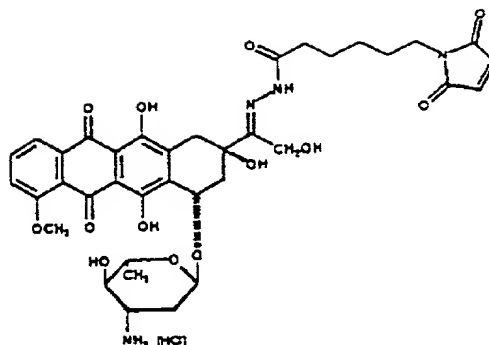
10

#### Example 4

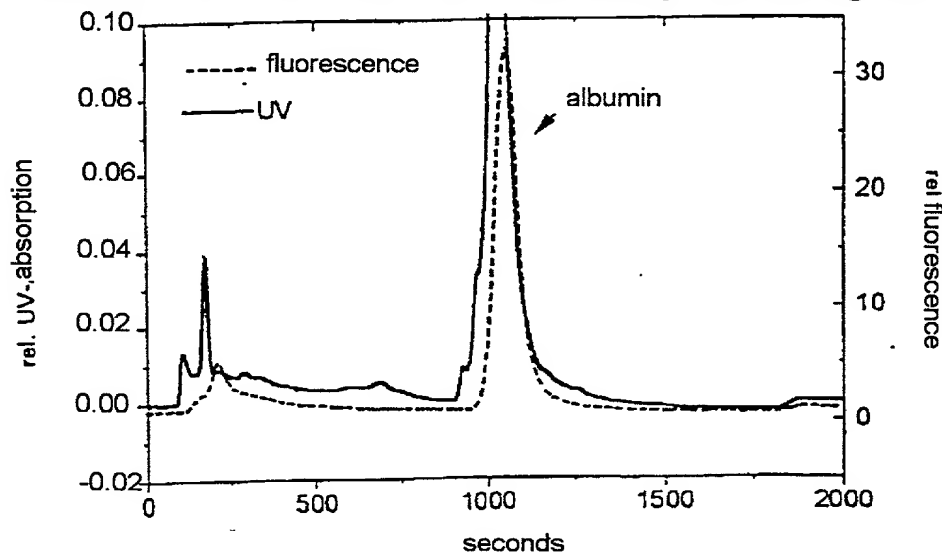
##### Binding of DOXO-EMHC to albumin in human plasma

1.6 mg of the 6-maleimidocaproic acid hydrazone derivative of doxorubicin (abbreviated to DOXO-EMHC)

15 having the following structural formula



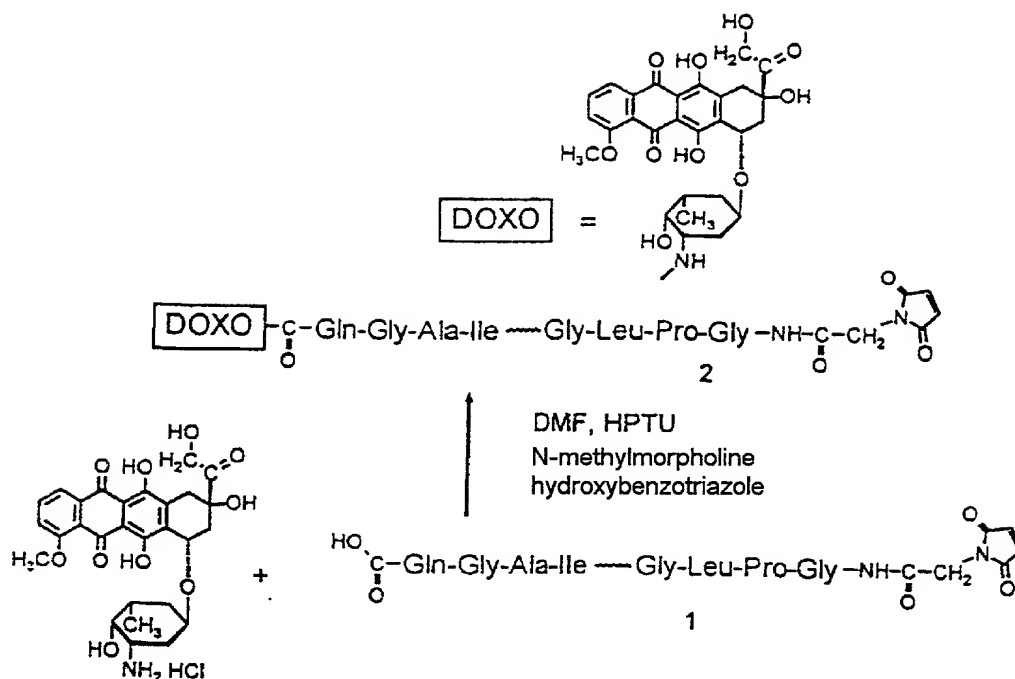
are dissolved, at room temperature, in 1.0 ml of phosphate buffer (0.15 M NaCl, 0.004 M sodium phosphate, pH 6.5) (2 000  $\mu$ M solution). When 250  $\mu$ l of this solution are incubated for 30 seconds, at 37°C, with 1.0 ml of human plasma and the sample is subsequently separated on a weak anion exchanger (from POROS®), it is found that the majority of the DOXO-EMHC is bound to albumin (see the following chromatogram):



#### Example 5

Binding of an MMP9-cleavable doxorubicin-maleimide-peptide derivative (2) to albumin following a one-minute incubation with human plasma

The doxorubicin-maleimide-peptide derivative (2) was prepared in accordance with the following reaction equation:



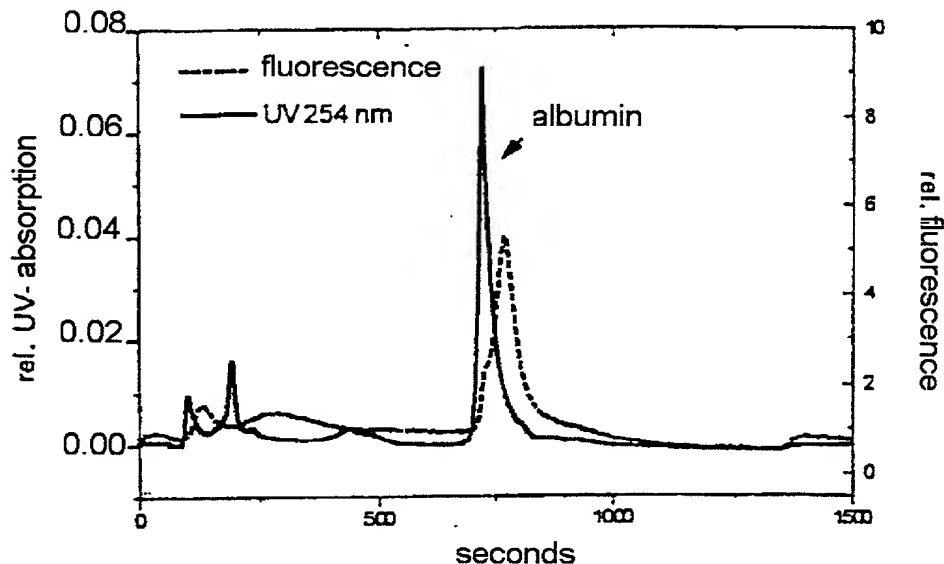
In this preparation, the maleimidoglycine-derivatized octapeptide Gln-Gly-Ala-Ile-Gly-Leu-Pro-Gly 1 (Mr 848, prepared by Bachem AG, Switzerland using solid-phase synthesis) is reacted with doxorubicin in accordance with the following protocol:

25 mg of 1 (as the trifluoroacetate salt), dissolved in 500  $\mu$ l of DMF, 33.5 mg of O-benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate (HPTU), dissolved in 200  $\mu$ l of DMF, 11.9 mg of hydroxybenzotriazole hydrate, dissolved in 100  $\mu$ l of DMF, and 16.2  $\mu$ l of N-methylmorpholine are added to a slightly turbid solution of 17.1 mg of doxorubicin in 3 ml of DMF and the mixture is subsequently stirred in the dark at RT for 18 h. DMF was removed under high vacuum and the solid taken up in 20 ml of methanol; this solution was then filtered and concentrated down to 1 ml in vacuo. After purifying on silica gel (ethyl acetate/methanol 2/1), 5 mg of 2 were obtained.

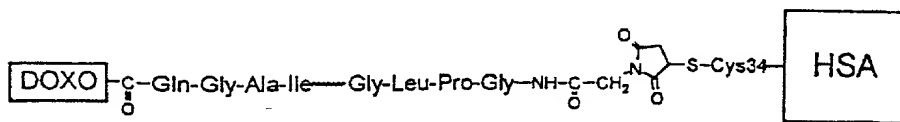
#### Incubation study using human plasma

1.4 mg of 2 (Mr 1374) are dissolved, at room temperature, in 1.0 ml of phosphate buffer (0.15 M NaCl, 0.004 M sodium phosphate, pH 6.5) (1 000  $\mu$ m

solution). When 300  $\mu$ l of this solution is incubated for 60 seconds, at 37°C, with 1.0 ml of human plasma and the sample is subsequently separated on a weak anion exchanger (from POROS®), it is found that the majority of 2 is bound to albumin (see following chromatogram) :



The peptide sequence Gln-Gly-Ala-Ile-Gly-Leu-Pro-Gly is recognized by the matrix metalloprotease MMP9 and cleaved between isoleucine and glycine. This was demonstrated by the following experiment: 200  $\mu$ l of a 100  $\mu$ M solution of the albumin conjugate of 2 having the following structure (abbreviated to HSA-2) :



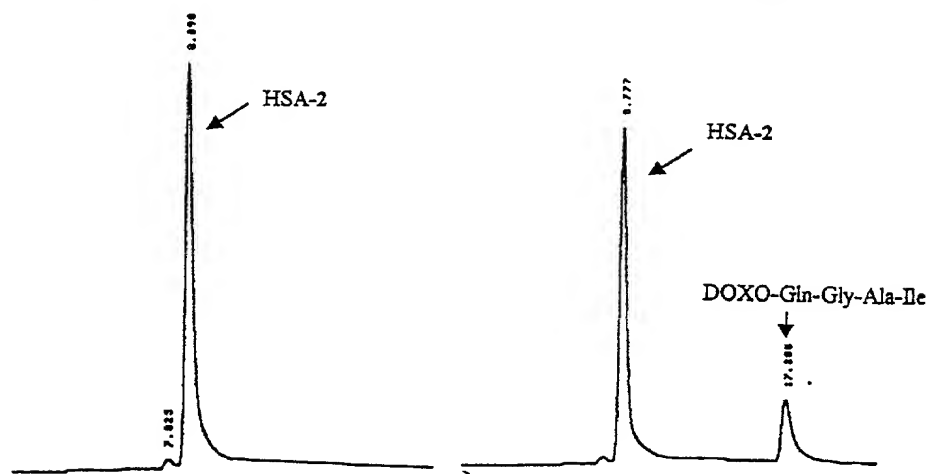
HSA = human serum albumin

which was prepared by the method described in German Patent Application A19926475.9, dated 10 June 1999, was incubated, at 37°C for 30 minutes, with trypsin/aprotinin-activated MMP9 (2 mU, from Calbiochem, Germany). The release of DOXO-Gln-Gly-Ala-Ile after this time is depicted in the following chromatograms. The figure shows the chromatogram of HSA-2 at  $t = 0$  (separation by means of HPLC exclusion chromatography using a Biosil 250 SEC column supplied

by Biorad, detection at  $\lambda = 495$  nm) and after having been incubated with activated MMP9 for 30 minutes.

t = 0 min

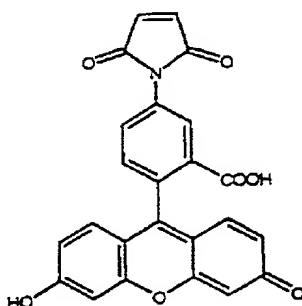
t = 30 min



5

### Example 6

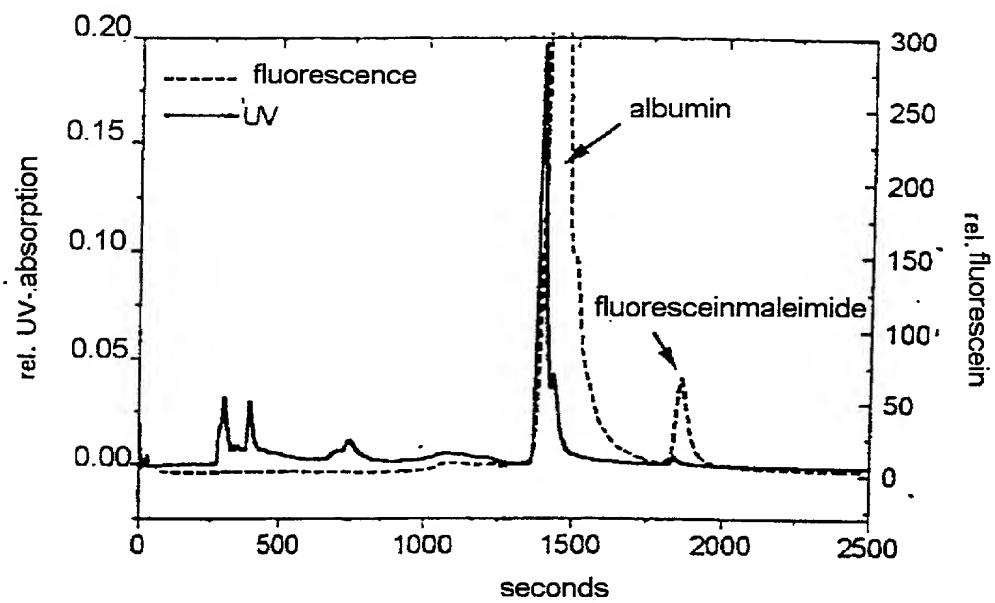
Binding of fluoresceinmaleimide to albumin in human plasma



10

After incubating 250  $\mu$ l of a 100  $\mu$ M solution of fluoresceinmaleimide solution (phosphate buffer = 0.15 M NaCl, 0.004 M sodium phosphate, pH 5.0) with 1.0 ml of human plasma for 5 minutes and subsequently separating the sample by means of gel filtration chromatography (Superdex<sup>®</sup>200, Pharmacia), it is found that the majority of the fluoresceinmaleimide is bound to albumin (see following chromatogram):

15



0990265 4301  
T00E11 99208660

Claims

1. Process for producing an injectable medicament preparation, which comprises a therapeutically and/or diagnostically effective substance which is dissolved in an injectable carrier liquid, characterized in that use is made, as the therapeutically and/or diagnostically effective substance, of a compound which consists of an active compound and at least one <sup>covalently</sup> protein-binding molecular residue which are linked by a spacer, in which the spacer, or the bond between the active compound and the spacer, can be cleaved hydrolytically or enzymically in the body in a pH-dependent manner.
2. Process according to Claim 1, characterized in that the spacer, or the bond between the active compound and the spacer, can be cleaved in the body, with the the release of the active compound or of a derivative of the active compound.
3. Process according to Claim 1 or 2, characterized in that the active compound is a cytostatic agent, a cytokine, an immunosuppressive agent, a virostatic agent, an antirheumatic agent, an analgesic, an antiinflammatory agent, an antibiotic, an antimicotic agent, a signal transduction inhibitor, an angiogenesis inhibitor or a protease inhibitor.
4. Process according to Claim 1, 2 or 3, characterized in that the active compound is selected from the group consisting of anthracyclines, nitrogen mustard derivatives, alkylating agents, purine or pyrimidine antagonists, folic acid antagonists, taxanes, camptothecins, podophyllotoxin derivatives, vinca



alkaloids, calicheamicins, maytansinoids or *cis*-configured platinum(II) complexes.

5. Process according to one of the preceding claims,  
5 characterized in that the diagnostically effective substance possesses one or more radionuclides, one or more ligands comprising radionuclides, one or more positron emitters, one or more NMR contrast agents, one or more fluorescent compound(s) and/or  
10 one or more contrast agents in the near IR range.
6. Process according to one of the preceding claims,  
characterized in that the protein-binding molecule is a maleimide group, a haloacetamide group, a  
15 haloacetate group, a pyridylthio group, an N-hydroxysuccinimide ester group, an isothiocyanate group, a disulphide group, a vinylcarbonyl group, an aziridine group or an acetylene group, which can, where appropriate, be  
20 substituted.
7. Process according to one of the preceding claims,  
characterized in that the spacer is an organic molecular residue, which contains an aliphatic  
25 carbon chain and/or an aliphatic carbon ring having 1-12 carbon atoms, some of which can be replaced with oxygen, and/or at least one aromatic moiety, which can, where appropriate, be substituted.
- 30
8. Process according to one of the preceding claims,  
characterized in that the bond between the active compound and the spacer or the protein-binding  
35 molecular residue contains at least one peptide bond.
9. Process according to one of the preceding claims,  
characterized in that use is additionally made of a carrier molecule.

09980266.11001

10. Process according to Claim 9, characterized in that the carrier molecule and the therapeutically and/or diagnostically effective substance are brought into contact ex vivo.
11. Therapeutically and/or diagnostically effective substance containing at least one active compound, characterized in that it possesses at least one protein-binding molecular residue which is linked to the active compound through a spacer, with the spacer, or the bond between the spacer and the active compound, being cleavable hydrolytically or enzymically in the body in a pH-dependent manner, and with the active compound not being a cytostatic agent.
12. Diagnostically effective substance containing at least one diagnostic agent, characterized in that it [lacuna] at least one protein-binding molecular residue which is linked to the diagnostic agent by means of a spacer, with the spacer, or the bond between the spacer and the diagnostic agent, being hydrolytically or enzymically cleavable in the body in a pH-dependent manner.
13. Use of the therapeutically and/or diagnostically effective substance according to one of Claims 1 to 10 for treating cancer diseases, virus diseases, autoimmune diseases, acute or chronic inflammatory diseases and diseases which are caused by bacteria, fungi or other microorganisms.
14. Diagnostic kit, which comprises the protein-binding diagnostically effective substance according to one of Claims 5 to 12, where appropriate a carrier molecule, and also pharmaceutically acceptable auxiliary substances, carrier substances and/or diluents.

15. Use of the diagnostically effective substance according to one of Claims 5 to 10 and 12, or of the diagnostic kit according to Claim 14, for detecting cancer diseases, autoimmune diseases, acute or chronic inflammatory diseases and diseases which are caused by viruses and/or microorganisms, and/or for detecting the carrier molecule and its distribution in the body.
16. Process for producing an injectable medicament preparation, which comprises a diagnostically effective substance which is dissolved in an injectable carrier liquid, characterized in that use is made, as the diagnostically effective substance, of a compound which comprises a diagnostic agent and at least one protein-binding molecular residue.
17. Process according to Claim 16, characterized in that the diagnostic agent and the protein-binding molecular residue are linked by a spacer.
18. Process according to Claim 16 or 17, characterized in that the bond between the diagnostic agent and the protein-binding molecular residue or the spacer are [sic] not cleavable.
19. Process according to Claim 18, characterized in that the diagnostic agent and the protein-binding molecular residue are linked to each other by an amide bond.

[illegible][illegible][illegible]

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
0	0.0000	0.0001	0.0002	0.0003	0.0004	0.0005	0.0006	0.0007	0.0008	0.0009	0.0010	0.0011	0.0012	0.0013	0.0014	0.0015	0.0016	0.0017	0.0018	0.0019	0.0020	0.0021	0.0022	0.0023	0.0024	0.0025	0.0026	0.0027	0.0028	0.0029	0.0030	0.0031	0.0032	0.0033	0.0034	0.0035	0.0036	0.0037	0.0038	0.0039	0.0040	0.0041	0.0042	0.0043	0.0044	0.0045	0.0046	0.0047	0.0048	0.0049	0.0050	0.0051	0.0052	0.0053	0.0054	0.0055	0.0056	0.0057	0.0058	0.0059	0.0060	0.0061	0.0062	0.0063	0.0064	0.0065	0.0066	0.0067	0.0068	0.0069	0.0070	0.0071	0.0072	0.0073	0.0074	0.0075	0.0076	0.0077	0.0078	0.0079	0.0080	0.0081	0.0082	0.0083	0.0084	0.0085	0.0086	0.0087	0.0088	0.0089	0.0090	0.0091	0.0092	0.0093	0.0094	0.0095	0.0096	0.0097	0.0098	0.0099	0.0100

[illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible]

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

\_\_\_\_\_  
(Application Number)

\_\_\_\_\_  
(Filing Date)

**U.S. Priority Applications**

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

\_\_\_\_\_  
(Applic. Serial No.)                      (Filing Date)                      (Status-patented/pending/abandoned)

\_\_\_\_\_  
(Applic. Serial No.)                      (Filing Date)                      (Status-patented/pending/abandoned)

**Power of Attorney**

I hereby appoint the following attorneys and patent agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Peter F. Felfe, Reg. No. 20,297; John E. Lynch, Reg. No. 20,940; Norman D. Hanson, Reg. No. 30,946; John A. Bauer, Reg. No. 32,554; Mary Anne Schofield, Reg. No. 36,669; James Zubok, Reg. No. 38,671; James R. Crawford, Reg. No. 39,155, Andrew Im, Reg. No. 40,657 and David Rubin, Reg. No. 40,314; my attorneys with full power of substitution and revocation.

Address all correspondence to: FULBRIGHT & JAWORSKI L.L.P.  
666 Fifth Avenue  
New York, New York 10103

(1) Felix Kratz 23/10/2009  
Full Name/Sole or First Inventor Signature Date

Residence: 79106 Freiburg DE X  
Germany

Post Office Address: c/o Klinik für Tumorbilogie  
Breisacher Straße 117  
79106 Freiburg /Germany

Citizenship: German ✓

(2)

---

Full Name/Second Inventor	Signature	Date
---------------------------	-----------	------

Residence: \_\_\_\_\_

Post Office Address: \_\_\_\_\_

Citizenship: \_\_\_\_\_